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TOMATO LEAVES

A POTENTIAL ALKALOID

AND STEROL SOURCE

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T. D. Fontaine, P. S. Schaffer, H. M. Doukas,
W. E. Scott, R. M. Ma, and V. A. Turkot*

Eastern Regional Research Laboratory**
Philadelphia 18, Pennsylvania
and
Floyd DeEds and R. H. Wilson
Western Regional Research Laboratory***
Albany, California
and
S. P. Doolittle
Horticultural Crops Research Branch
Agricultural Research Service
Beltsville, Maryland

INTRODUCTION

Tomatine, a new crystalline glycosidal alkaloid, first isolated from the leaves and stems of the wild tomato species *Lycopersicon pimpinellifolium* (Jusl.) Mill. in the Eastern Utilization Research Branch laboratories (9),**** is composed of an aglycone portion, tomatidine (9), and a tetrasaccharide moiety consisting of two molecules of glucose and one each of xylose and galactose (23). It also is present in the cultivated tomato *L. esculentum* Mill. Tomatine has been found to have fairly high *in vitro* antifungal activity against certain fungi parasitic on animals, but is much less effective against fungus plant pathogens (8, 14, 15). The possible role of tomatine in resistance to fusarium wilt disease of tomato plants caused by the fungus *Fusarium oxysporum* f. *lycopersici* Snyder & Hansem has been considered (13, 14). Rutin, a pharmaceutical of considerable commercial importance (12), was isolated from tomatine concentrates (10) and was found to exert an antagonistic effect on the *in vitro* antifungal activity of tomatine toward *Candida albicans* (22).

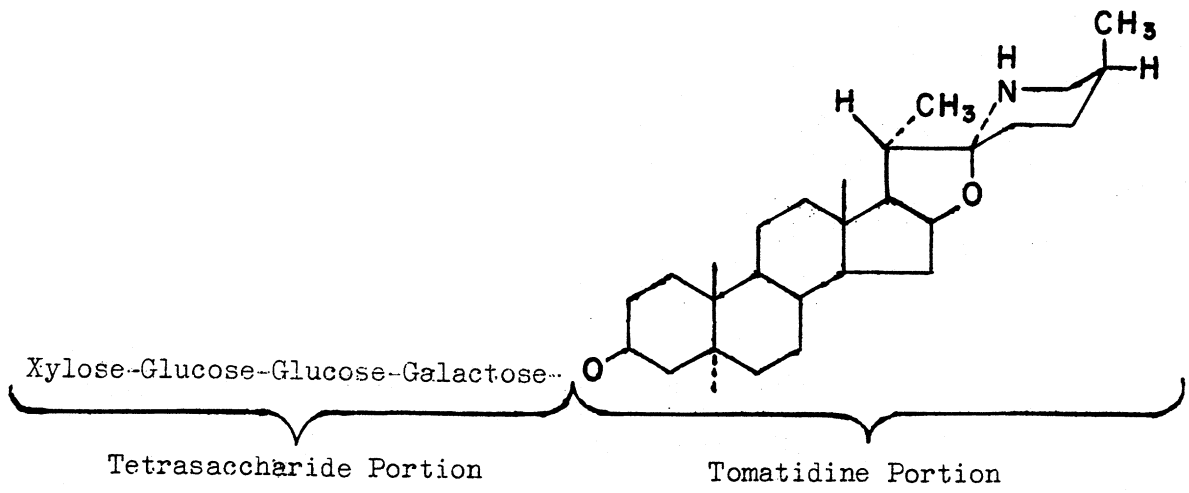
* PRESENT ADDRESS. BIOLOGICAL SCIENCES BRANCH, AGRICULTURAL MARKETING SERVICE, U. S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND.

** A LABORATORY OF THE EASTERN UTILIZATION RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE.

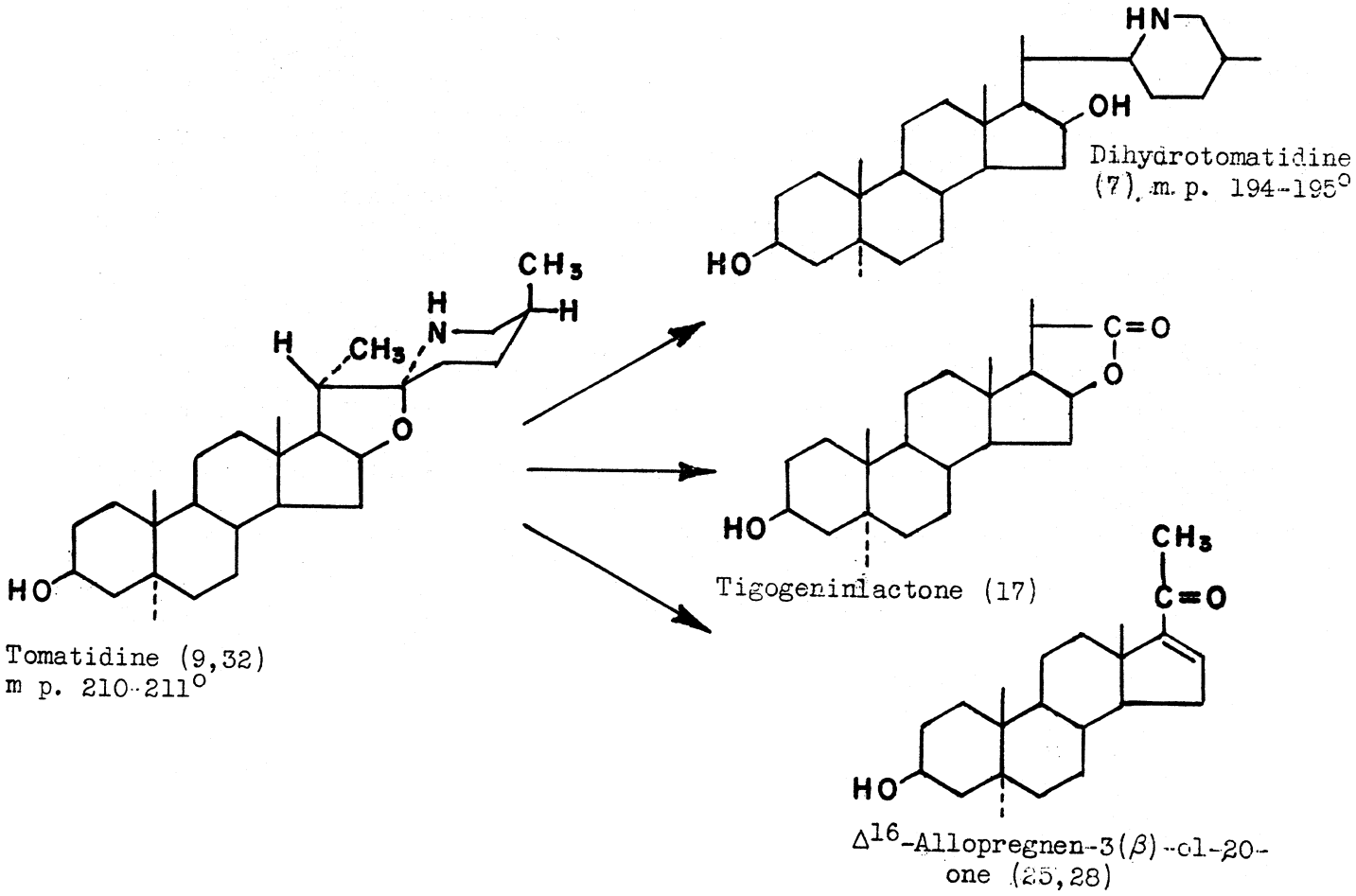
***A LABORATORY OF THE WESTERN UTILIZATION RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE.

****NUMBER IN PARENTHESIS REFERS TO LITERATURE CITED, PAGE 22.

Tomatine ($C_{50}H_{83}O_{21}N$), as a result of our own and other investigators work (7, 9, 16, 17, 18, 20, 21, 23, 28, 32) can now be assigned the following structure:



Tomatidine ($C_{27}H_{45}O_2N$) is prepared from tomatine by acid hydrolysis (9). Tomatidine has been degraded to the following compounds:



Thus tomatidine may at some future date be of value as a precursor of the sterol Δ^{16}- allopregnen-3 (β)-ol-20-one which in turn can be converted to sex and other type hormones (2). Tomatidine has been synthesized (32) from a plant sapogenin, neotigogenin, by steps that have led to assignment of structure as depicted above, thus demonstrating the close chemical relationship between some steroidal sapogenins and steroidal alkaloids. A number of derivatives of tomatidine and dihydrotomatidine have been prepared in the Eastern Regional Research Laboratory. These are being tested for antifungal and pharmacological properties and will be reported later.

On the basis of our published work, Drogaco Industria Quimica S.A.,[+] Buenos Aires, Argentina, is now preparing tomatine, offering it for sale and recommending it for topical use in the treatment of fungus diseases of the skin (3). Pharmaceutical firms in the United States have been interested in a number of possible uses for tomatine and derived products.

Kuhn *et al.* (19) have reported high yields of tomatine from dried leaves of *L. esculentum* var. *pruniforme* Voss (up to 5 percent) and a number of other species and varieties grown in Germany. We have not found such high tomatine yielding varieties in our limited tests of tomato plant species and varieties in the United States; our yields of crystalline tomatine from dried leaves varied from 0.2 to 1.5 percent depending upon the species, variety and age of plant. Oven dried leaves and fresh leaves yielded essentially the same amount of crystalline tomatine. In nearly all of our tests, the highly fusarium wilt resistant species, *L. pimpinellifolium* (Red Currant), was found to contain the highest tomatine content.

Prokoshev *et al.* (27) have reported an average of 0.63 mg. tomatine/100 g. dry leaves of *L. esculentum* var. *vulgare* Bailey in July, 0.81 mg. in August and 0.59 mg. in September; *L. esculentum* var. *validum* Bailey averaged 0.82, 0.93, and 0.48 percent, respectively. The highest tomatine level (1.57 percent) was found in *L. pimpinellifolium*, which is in agreement with our findings. On the other hand, they report that *L. esculentum* var. *pruniforme* Voss contained only 0.62 percent tomatine which is in marked contrast to the 5.0 percent value reported by Kuhn *et al.* (19). Galinovsky and Wagner (11) have reported values for tomatine content of tomato leaves in line with our findings. Tomatine has been reported to be present in tomato fruit (27), the highest concentration occurring in green tomatoes, reaching a minimum in pink fruit and rising slightly in completely ripe tomatoes. Brink and Folkers (5) have isolated tomatidine in very low yield, from the roots of the Rutgers variety(*L. esculentum*).

Over 650,000 acres were planted in tomatoes in the United States in a peak year, 1951. Of this total, approximately 225,000 acres were for the fresh produce market and 425,000 acres for processing. There are approximately 2,500 to 3,000 tomato plants per acre when grown for fresh produce without pruning and staking and approximately the same number of plants per acre when grown for processing. Not

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all of this crop would be suitable for the production of tomatine at the end of harvest because leaf spot diseases often cause loss of much of the foliage. In some areas, however, particularly in the semi-arid West where leaf spot diseases are not severe and the fields are irrigated, tomato vines have a lush growth at this time. It might be desirable to grow tomato plants specifically for the purpose of preparing tomatine if a demand for this material develops and, if so, the high yielding varieties reported by Kuhn *et al.* (19) should be reinvestigated. In one of our experimental plantings, it was found that the Rutgers variety at the time of flowering, but with no large fruit formed, yielded approximately 300 grams dry weight for the above-ground portion of each plant, of which approximately 60 percent (180 grams) was leaf meal containing most of the tomatine. On the basis of 10,000 tomato plants per acre grown to the above size, it is estimated that two tons of dried leaf meal would be obtained, which if it contained 1 to 5 percent tomatine would yield from 40 to 200 pounds of tomatine or from 16 to 80 pounds of tomatidine per acre.

We have carried out, over a number of years, investigations designed to determine the variation of tomatine in leaves and stems of *L. pimpinellifolium* and several commercial varieties of *L. esculentum* at different stages of maturity, to develop laboratory and pilot plant methods for drying tomato vines, separating leaf and stem meals, extracting plant materials and recovering tomatine. The primary objectives of this paper are to present new unpublished information on the above mentioned subjects and to summarize the antifungal tests and pharmacological and toxicological findings on tomatine and tomatidine.

EXPERIMENTAL

Isolation of Crystalline Tomatine--Laboratory Method.--The method now used to study the distribution of tomatine in tomato plants is outlined in the following steps: (a) Dry tomato plants at about 85°C. in a forced-draft oven. (b) Extract 100 grams of dried ground tomato plant material with 1500 ml. boiling 95 percent methanol with stirring for 30 minutes, pour off the extract and re-extract the marc twice more for 15 minutes each with an equal volume of 95 percent methanol; combine the methanol extracts and concentrate in an all-glass vacuum evaporator until the volume is reduced to approximately 100 ml. (c) Acidify the 100 ml. methanol concentrate with 100 ml. 0.1 N HCl and extract with two 100 ml. portions of benzene to remove lipids and chlorophyll. (d) Precipitate the crude tomatine from the benzene extracted solution by adjusting to pH 10 with sodium hydroxide solution. Collect the precipitate by centrifugation. (e) Dissolve the precipitate in 50 ml. 0.1 N HCl and extract out tomatine · HCl with butanol saturated with water. (f) Concentrate the butanol extract in the vacuum evaporator and add sufficient water to remove most of the butanol by distillation. Precipitate tomatine from the aqueous solution by the addition of sodium hydroxide solution to pH 10. Collect the precipitate by centrifugation. (g) Wash the precipitate with a small amount of acetone to remove a trace of color and then crystallize tomatine from a minimum amount of hot 75 percent ethanol.

Tomatine may be purified further by re-extracting the crystalline product with a low-boiling hydrocarbon solvent to remove traces of waxy materials, followed by hot benzene extraction to remove traces of tomatidine and color. This product is then recrystallized from a minimum amount of 95 percent ethanol. Our best samples of tomatine have a melting point of 290-291°C. (evacuated sealed capillary tube) but, in general, most purified samples melt around 285°C. Tomatine is readily soluble in aqueous-acid solutions, is fairly soluble in 95 percent methanol

and ethanol, 1,4-dioxane and propylene glycol at room temperature, but is highly insoluble in water at alkaline pH values and in benzene, chloroform and hydrocarbons.

Conversion of Tomatine to Tomatidine: Tomatine is hydrolyzed by boiling 2 N hydrochloric acid solution, yielding a fairly insoluble portion, tomatidine hydrochloride, and a supernatant solution rich in reducing sugars. Tomatidine hydrochloride is recovered and converted to the free base, tomatidine. These steps may be illustrated by a typical experiment.

Partially purified tomatine (100 grams) was placed in a 3-neck round bottomed flask equipped with a mechanical stirrer and reflux condenser; 2500 ml. of 2 N hydrochloric acid solution was added and the solution refluxed for three hours. After cooling to room temperature, the insoluble precipitate, tomatidine hydrochloride, was recovered on a sintered glass funnel, then washed on the funnel with a small amount of acetone to remove some green color. A yield of 39.5 grams of tomatidine hydrochloride or 90 percent of theoretical (43.7 grams theory, assuming starting material pure), was obtained.

Tomatidine hydrochloride (40 grams) was suspended in 1500 ml. of 75 percent methanol in a 2-neck round bottomed flask equipped with stirrer, to which was added 40 ml. of concentrated ammonium hydroxide. The mixture was refluxed, with stirring, until all the material had dissolved. Tomatidine crystallized on cooling in an ice bath and was collected on a sintered glass funnel. Additional amounts of tomatidine were recovered on concentrating the filtrate. Sodium hydroxide, instead of ammonium hydroxide, could also be used to convert tomatidine hydrochloride to tomatidine.

The first crop of crystalline tomatidine thus obtained melted at 203-205°C. but after further purification, by dry benzene elution from an Al_2O_3 column, melted at 210-211°C.

Other Experiments--In the processes used to extract tomatine from tomato plant parts, our usual procedure involved drying the plant material and then extracting it with hot methanol or water. In a few experiments, we extracted fresh undried tomato leaves. Thirty-six pounds of green Rutgers tomato leaves were obtained from the greenhouse and were divided into three 12-pound lots. These three batches were assayed as follows:

1. Twelve pounds of leaves were dried in an oven in the usual manner. The dried leaves were ground and extracted with hot methanol and the tomatine isolated by the regular laboratory procedure. The yield of tomatine on a dry leaf basis was 0.69 percent.

2. Twelve pounds of fresh green leaves were ground in a food chopper and then extracted with hot methanol. The aqueous methanol extract was concentrated under reduced pressure and tomatine recovered by the regular laboratory procedure. The yield of recovered tomatine on a dry leaf basis was 0.73 percent.

3. Twelve pounds of green leaves were ground in a food chopper and extracted with two portions of hot water at pH 5. The water was pressed out of the marc on a fruit press. The filtrate, in a large jar, was made alkaline to

pH 9-10 with dilute NaOH. After standing overnight, approximately 90 percent of the supernatant solution was decanted and discarded. The lower fraction was centrifuged and the precipitate was dried in the oven. This dried alkaline precipitate was extracted with methanol and the tomatine recovered. The yield on a dry leaf basis was only 0.36 percent. This experiment indicated a loss due either to incomplete extraction of tomatine or incomplete recovery of the alkaline precipitate.

Another batch of fresh green leaves was separated into three equal lots and extracted with water under varying conditions.

4. Boiling water was poured directly over the chopped leaves in the fruit press and permitted to run out immediately. It was hoped that this might remove most of the chlorophyll, so that the second leach would yield a better product. The water extract was made alkaline and the alkaline precipitate was recovered, dried and extracted with methanol. The crude tomatine was colored green and had to be purified with charcoal. A second and third leach of one hour each was given the marc and the tomatine recovered as above. The total yield of tomatine on a dry leaf basis was 0.60 percent.

5. A batch of chopped green leaves was boiled for 1/2 hour in water. This procedure, upon processing, gave a greenish-yellow alkaline precipitate that was not too difficult to purify. A second and third hot water leach of one hour each was made on the same marc. The total yield of tomatine was 0.61 percent. The third leaching could be eliminated without appreciable loss of tomatine.

6. The third batch was cooked for two hours, and repeated a second and third time. These extracts yielded an alkaline precipitate that was yellow and settled readily so as to make separation easy. The total yield of tomatine was 0.84 percent. The third leaching could be eliminated without appreciable loss of tomatine.

The results of experiments nos 4-6 suggested that prolonged extraction of fresh tomato leaves was advantageous in increasing the yield of tomatine.

In another experiment, fresh leaves of 35 day old Southland variety of tomato were divided into two fourteen pound lots. One lot was dried, ground and extracted with methanol and recovered in the usual laboratory manner. The yield of tomatine was 0.90 percent. The second lot of leaves was put through a food chopper and then extracted twice, two hours each time with boiling water. The alkaline precipitated material, when extracted with methanol, yielded 1.0 percent tomatine. It would appear that if fresh leaves are extracted under proper conditions, the yield of tomatine can be increased appreciably and compares favorably with the methanol extraction process.

Our earlier method for the preparation of tomatine (9) did not lend itself to even a quantitative approximation of the amount in tomato plants. The crystallization of tomatine was hindered by the presence of impurities that formed gels. A procedure, which has since been abandoned for a better method, was developed whereby tomatine was given a preliminary purification prior to attempting to crystallize it. The purification step is recorded here because it is unique and may be of possible value to other investigators working on alkaloids. Crude tomatine was obtained by adjusting an aqueous extract of tomato leaves to pH 10 with alkali

and collecting the precipitate by centrifugation (9). This material (dried or moist) was dissolved in a minimum amount of hot alkaline 70 to 80 percent aqueous-1,4-dioxane, methanol, or ethanol etc. One and one-half parts of hot water (about 90°C.) was then added rapidly with vigorous stirring. At first, upon the addition of the hot water, a precipitate was formed which was then almost completely redissolved, whereupon crystallization of tomatine took place. The crystals were highly birefringent and when stirred formed glistening streams. Heating and stirring were continued for a time and then the hot solution was centrifuged for a short time. The crystalline tomatine settled rapidly and the major impurities remained soluble in the hot supernatant solution which was poured off. Tomatine then recrystallized readily from hot 75 percent alcohol.

Variation in Yield of Tomatine with Species and Age of Plant--Two species of tomato plants were grown under field conditions in order to establish the age at which plants gave a maximum yield of tomatine. Red Currant tomato plants (*L. pimpinellifolium*) which are highly resistant to fusarium wilt disease, were grown from seeds planted in a greenhouse at Beltsville, Maryland, on April 8, 1952, and were transplanted to the field on May 29. Plants of the commercial variety Rutgers, which is fairly resistant to fusarium wilt disease, were grown from seeds planted in the field at Tifton, Georgia, on March 20 and pulled and shipped to Beltsville, Maryland, for field planting on May 22. Both species were fertilized well in order to obtain good foliage before fruit set. The two species of plants were sampled at the ages shown in Table I which are based on time of planting seed. Tomatine content was determined by the procedure outlined in the previous section.

The maximum tomatine content in Red Currant leaves (Table I) was found when the plant was growing rapidly and flowering but had not set much fruit (104 days). This age also gave the highest yield for the entire plant. One week later when the plants had set fruit and had doubled in weight, the tomatine content of the leaves and stems had decreased markedly. In this experiment, Rutgers leaves at the time of harvest (130 days) were found to contain more tomatine than Red Currant leaves (118 days). At the time of harvest both species had set considerable fruit.

Drying of Tomato Vines in Heil Direct-Fired Rotary Drier--The tomato plants grown in the field at Beltsville, Maryland, were cut with a field mowing machine, gathered with a hay rake and loaded on open-stake bodied trucks and transported approximately 150 miles to the Eastern Regional Research Laboratory. Two truckloads of Rutgers tomato vines were delivered. The vines appeared to be in good condition, not molded or spoiled. Most of the tomatoes had been picked off the vines before harvest and the few remaining were sorted out and did not enter the cutter and drier.

Since the leaves of the tomato plant contain most of the tomatine, it was necessary to dry the plant by a method that would not scorch the leaves. This was done by a fractional drying process (1). It consisted in chopping the plant in a rotary forage cutter set to give a 3/4" nominal cut and feeding the material into a rotary, direct-fired, alfalfa drier with its inlet and exhaust temperatures adjusted so that the leaves become dry and brittle but the stems remain moist and

Table I

Yield of Tomatine (By Laboratory Method) from Two Species of Tomato Plants at Different Ages under Field Conditions

Tomato plants	Age of plants	Average dry wt. per plant			Yield of tomatine per plant				
		leaves	stems	total	leaves		stems		total
	days[1]	g.	g.	g.[2]	mg.	%	mg.	%	%
Red currant	90	31.0	31.0	62	223	0.72	90	0.30	0.51
" "	97	88.2	52.5	140.7	987	1.12	52	0.10	0.74
" "	104	95.0	71.5	166.5[3]	1170	1.23	346	0.49	0.91
" "	111	212.0	142.5	354.5	1748	0.83	292	0.20	0.55
" "	118[4]	205.5	191.0	396.5	1391	0.68	573	0.30	0.49
Rutgers	109	41.0	44.8	85.8	396	0.90	84	0.19	0.53
"	116	78.7	73.0	151.7	425	0.54	164	0.23	0.39
"	123	121.5	116.0	237.5	823	0.70	63	0.05	0.38
"	130[4]	163.0	123.0	286.0	1440	0.89	49	0.04	0.52

1/ From time of planting seed.
2/ Exclusive of fruit and roots.
3/ Plants were flowering but had not set fruit.
4/ Plants harvested, transported to the Eastern Regional Research Laboratory and dried in a direct-fired rotary drier (See section on drying).

tough. The dry leaves were detached from the moist stems by the fans in the drier through which the material passed. By means of a vibrating screen equipped with a 7-mesh sieve the leaf fraction was separated from the stem fraction. The dried tomato leaf meal passed through the sieve while the moist stems passed over it. The stems were collected for further drying.

When the drying operations reached equilibrium conditions all pertinent data were recorded. These data are given in Table II.

The tomatine content of tomato stems is generally quite low as compared to the leaves and in actual practice they would probably be discarded. In our work, however, it appeared desirable to dry the stems. The stems (+7-mesh) were still quite wet after this first drying, being now at 61.2 percent moisture content. Some of them were put through the drier again without recutting. These, on leaving the drier, did not feel quite dry enough, so feeding was stopped. Analysis later showed the moisture content of these stems to be 13.1 percent, probably low enough to keep without spoiling. The remaining wet stems were spread out evenly over a large area of the drier building floor before leaving them overnight to help prevent spoilage. Apparently there was no appreciable fermentation or "working" in them overnight.

A Fitzpatrick[+] mill was set up the next morning to chop the remainder of the wet stems before drying them. This machine, a type of hammer mill, has fixed, forward-curved, sharp-edged, stainless steel knives. A 1" perforated screen was used in the mill and a good cutting job was done, most of the thicker stems being split longitudinally. Drying of this material proceeded quite satisfactorily; data are given in Table II.

A week later two truckloads of Red Currant tomato vines were delivered. These vines had retained a large number of their tiny fruit, a few red but mostly green tomatoes about 1/2- to 3/4-inch in diameter. Many tomatoes fell off the vines during handling but an appreciable number entered the cutter and drier. They did not appear to affect the drying adversely. This load was somewhat larger than the first lot of Rutgers vines. Also this load contained some portions which had obviously undergone spoilage, since they were warm, gray-brown, and slimy to the touch. This part was possibly 5 percent of the total and, because of the difficulty of separating it, was dried along with the rest.

The vines were fed into a Fox cutter with 3/4" cut as in the previous run and then directly to the drier feed belt. The product consisted of dry meal through a 7-mesh screen, and wet stems over the screen. Twenty-two bags of meal with a net weight of about 870 pounds were produced; also one bag (46 pounds) of meal redried at 350°F. inlet temperature because it had come out too wet. A composite sample from two of the more "moist" bags of meal analyzed 9.6 percent moisture.

A 10-minute equilibrium or "test" run was included during the drying to obtain data, which are summarized in Table II. The "equilibrium" meal analyzed 8.4 percent moisture.

Table II

Tomato Vines Dried in Heil Rotary Drier, July-August 1952

Feed Material	Type of cut	Feed, lb/hr.	Rate lb. mfb per hr.	$\%H_2O$ in feed	Total feeding time hr.	Temperatures °F. inlet	Temperatures °F. outlet	Product rates -7-Mesh lb/hr	-7-Mesh $\%H_2O$	-7-Mesh lb.mfb/hr.	+7-Mesh lb/hr	+7-Mesh $\%H_2O$	+7-Mesh lb.mfb/hr.	Total product lb/hr as-is	Total product lb/hr mfb
Rutgers[a] vines	Fox 3/4"	2540	521	79.5	2.5	890	257	214	5.75	202	654	61.2	254	868	456
Rutgers[a] wet stems	not recut	900	350	61	0.6	600	315	-	-	-	-	13.1	-	-	-
Rutgers[b] wet stems	Fitz-mill	970	420	56.8	1.0	570	273	-	4 05	-	-	8.25	-	-	-
Red Currant[c] vines	Fox 3/4"	2180	483	77.85	4.0	850	257	207	8.40eq. 9.60 non-eq.	190	434	52.2	207	641	397
Red Currant[d] vines	not recut	1400	736	47.4	1.4	640	260	-	3.80	-	-	8.20	-	-	-

a, b, c and d refer to runs Nos. V18K43, V18K44, V18K45 and V18K46, respectively.

The following day the wet stems, which had been spread out on the floor overnight and now analyzed 47.4 percent moisture, were put through the drier. Since these stems were more slender than the Rutgers variety, it was decided not to recut them first, and the drying proceeded satisfactorily without the recutting. Data are given in Table II.

In all of the dried products obtained from both the Rutgers and the Red Currant tomato vines, there was none that appeared to be excessively scorched or burned. Likewise all products appeared sufficiently dry to keep in storage without spoiling. A summary of the amounts of the various dried fractions obtained is given in Table III. It must be emphasized that the drying results represent only two experiments. If equipment is available the fresh plant material could be extracted, of course, without drying. Tomatine is not destroyed during the drying process and, therefore, drying plant material before it is extracted has several advantages over processing the fresh plant. The more important advantages are as follows:

(a) It permits the extraction plant to operate throughout the year, or at the convenience of the processor.

(b) The capital investment for the smaller plant would be very much less than for one that extracts fresh material for only a few weeks.

(c) More solvent per pound of final product recovered would be required for extracting fresh material, hence, greater solvent losses.

(d) The manufacturing cost per pound of final product would be greater when fresh plant is used.

(e) A drier could be moved where the tomatoes are grown. An extraction plant (for fresh material) should be located near the source of raw material to prevent spoilage of plant and reduce haulage expense; this would limit the amount of available raw material.

Table III

Summary of Amounts of Dried Tomato Vine Products from Heil Drier, July-August 1952

Run No.	Net wt. leaf meal (-7-mesh)	Net wt. stems (+7-mesh)	Net wt. stems (-7-mesh)
	pounds	pounds	pounds
	A. Rutgers Tomato Vines		
V18K43	500	125	67
V18K44	---	263	165
Totals	500	388	232
Total dried product: 1120 pounds			
	B. Red Currant Tomato Vines		
V18K45	910	---	---
V18K46	---	720	324
Totals	910	720	324
Total dried product: 1954 pounds			

Pilot Plant Production of Tomatine.--The two processes described in this report have not been subjected to cost analyses and are intended, therefore, only to point the way to development of economical processes for the production of tomatine. The solvent extraction procedure using hot methanol or ethanol might have some advantage over the aqueous process depending on whether or not chlorophyll, lipids, etc. were to be recovered. In the particular experiments reported here, the primary objective was to obtain sufficient tomatine for chemical investigations and pharmacological testing.

Aqueous Process.--Preliminary pilot-plant runs were made using approximately 100 lb. batches of dried tomato plant parts to get an estimate of tomatine obtainable. The plant material was mixed with two gallons water/lb. dry material in a 300 gallon steam jacketed kettle fitted with a stirrer and a false bottom with screen. The pH was adjusted to 5.0-5.5 with acid and then the temperature was raised to 90-95°C., with continuous stirring, and maintained for 30 minutes. Stirring and heating were then discontinued for 15 minutes to allow the marc to settle. The eluate was pumped from the bottom of the tank to a settling tank. The marc was re-extracted with hot water (1 gallon/1 lb. original material) and the eluates combined. (In subsequent runs the 2nd eluate was used to extract a new batch of dried plant material.) After settling out most of the fines, the eluate was pumped from the top into a precipitating tank equipped with a stirrer. Concentrated sodium hydroxide solution was added, with stirring, to bring the extract to pH 9.5 to 10.0. A precipitate formed which settled rapidly from the warm solution. Approximately 2/3 of the supernatant solution could be pumped off when the precipitate was allowed to settle, otherwise the entire mixture was fed into a Sharples+ centrifuge at a rate which collected the gelatinous precipitate. The centrifugate was discarded. The dark brown precipitate was removed from the centrifuge bowl and dried in a forced draft oven at 90°C. The dried product was extracted batchwise with boiling methanol and the combined methanol extracts concentrated until tomatine began to precipitate. The solution was heated to near boiling and sufficient methanol added to completely dissolve any precipitate. On cooling and refrigerating, tomatine crystallized. This product was collected and the mother liquor concentrated to yield a second crop of tomatine. Results of these experiments, using various dried plant fractions, recorded in Table III, are given in Table IV. We are unable to account for the low yield of tomatine from Red Currant leaves in this particular run. The most logical reason is that in the centrifugation step the precipitate was not adequately collected.

Table IV

Yield of Tomatine from Tomato Plants Dried in a Direct-Fired Rotary Drier - Aqueous Process

Plant part	Quantity processed lbs.	Tomatine obtained lbs.	Yield tomatine %
Dry Red Currant stems	105	0.046	0.044
Dry Rutgers stems	100	.037	.037
Dry Rutgers stem meal	125	.077	.062
Dry Red Currant stem meal	100	.143	.143
Dry Rutgers leaf meal	111	.611	.550
Dry Red Currant leaf meal	120	.325	.269[a/]

a/ See text for possible explanation of low value. The alternative large scale Solvent Process yielded 0.68 percent tomatine which was the same as obtained by the small scale laboratory method on the plant material at the time of harvest (Table I).

Solvent Process.--Extraction of dried tomato leaves and stems with hot methanol was conducted in a hazardous operation building. The equipment was of stainless steel construction, with the vapor condenser vented to the outside. The equipment consisted essentially of a stainless steel percolator of approximately 200 gallon capacity, equipped with triple screens at the bottom, a mechanical stirrer, a liquid pump, sight gage, top and bottom pipe openings for the percolating solvent, a top opening and bottom opening for adding and removing the plant material The percolator was connected by piping to a slightly larger evaporator, equipped with both a steam jacketed bottom heater and a calandria. The top of the evaporator was equipped with a packed distilling column connected at the top to a large water cooled condenser. The top of the condenser was vented to the outside so that any uncondensed vapors were removed from the building. The condensate was returned to the percolator to be re-used in the extraction. Air was withdrawn at the floor level by the ventilating system.

Tomato plant parts, dried and ground to pass a 1/4 inch screen, were added to the percolator. Approximately 100 lb. samples were used. After the opening was tightly sealed, methanol containing about 10 percent water was pumped into the percolator to adequately cover all of the plant material. The liquid pump was then operated to recycle the methanol extract from the bottom of the percolator through a calandria operating at 18 lbs. steam pressure. When the temperature of the methanol reached 130°F. (54°C.) some of the methanol was by-passed into the evaporator. Here it was heated by pumping through a calandria operating at 8-10 lbs. steam pressure. The methanol vapors ascended through the packed column to the condenser. The condensate returned through a rotameter into the top of the percolator. The rate of evaporation was held to two gallons a minute to avoid flooding the condenser. In this manner, the system was operated with constant removal of extract while the liquid level in the percolator remained the same. The extraction was contained for eight hours. After all of the free methanol in the percolator was pumped into the evaporator, steam was passed through the extracted marc to remove the residual methanol. This methanol was also added to that in the evaporator. The hot extracted marc was then removed and discarded. After reloading with a fresh batch of plant material, the methanol in the evaporator was distilled into the percolator. The black sludge residue in the evaporator, containing the tomatine and other plant constituents, was removed through a valve opening in the bottom of the evaporator.

The black sludge was filtered through glass wool which removed some chlorophyll-lipid materials. The filtrate was diluted with an equal quantity of water and adjusted to pH 10 with sodium hydroxide solution. Since this solution clarified very slowly, it was run through an electrically driven Sharples centrifuge and the alkaline precipitate was recovered from the centrifuge bowl. This alkaline precipitate was dried in a forced draft oven at 90°C., yielding a somewhat sticky black mass which lacked the brittleness of a similar product derived from the water extraction process. An attempt at recrystallization from methanol proved unsuccessful. The black alkaline precipitate was dispersed with stirring in water and then dissolved by adjusting to pH 5.0 with acid. The tomatine was reprecipitated by the addition of alkali to pH 10. After standing overnight in a glass container, the clear dark supernatant solution was syphoned off and discarded. This process was repeated several times, until the alkaline precipitate had only a yellow brown color. After the last decantation, the partially purified tomatine was recovered by filtration on a Buchner funnel. The precipitate, after

drying at 80°C. in a mechanical convection oven, was a gray colored material, readily crystallizable from hot methanol. One hundred grams of the partially purified product was dissolved in 3500 ml. of hot methanol in a round bottomed flask, equipped with a mechanical stirrer. After filtering and cooling, crystalline tomatine was recovered.

Previous batches of tomatine, prepared by the hot water extraction method but crystallized from methanol had been found to contain as much as 10 percent of a waxy hydrocarbon when washed with n-hexane. The waxy hydrocarbon may have been responsible for some of our earlier problems encountered in crystallizing tomatine and obtaining good analyses. Tomatine, prepared by the methanol extraction method, was found to contain essentially no hydrocarbon.

Three different plant parts, obtained from two species of tomatoes, were extracted. Plant parts from the same plants had previously been extracted by the hot-water method, so a comparison of yields by the two processes can be made. Below is a summary of the yield of tomatine, after one crystallization from methanol:

Species & plant part	Yield.--methanol extn. %	Yield, water extn. %
Red Currant leaf meal	0.68	0.27
Rutgers stem meal	0.04	0.04 0.06
Red Currant stem meal	0.10	0.14

The yield of tomatine from Red Currant leaf meal was of the same order as had been indicated to be present from controlled small scale laboratory experiments, (See Table I), when the alcohol extraction method was used. Additional work will have to be done to establish the superiority of the alcohol extraction process over the water method because laboratory experiments had indicated much closer comparisons than recorded above.

BIOLOGICAL ACTIVITY OF TOMATINE AND DEGRADATION PRODUCTS

Plant Disease Aspects.--A consideration of possible chemical or biochemical factors that might contribute to some degree to disease resistance in plants led us to test tomato plant extracts for antifungal activity (13,14). The ultimate result was the isolation of a crystalline antifungal agent, tomatine, from tomato plants. Tomatine was found to inhibit the specific fungus, *Fusarium oxysporum* f. *lycopersici*, responsible for fusarium wilt in tomato plants. To date, the possible role of tomatine in relation to the degree of disease resistance of tomato plants to fusarium wilt has not been settled. We have found tomatine in both wilt susceptible and resistant tomato plants, and in infected plants that survived the infection, but not in plants that succumbed. It is necessary to point out, however, that we were employing an antifungal assay procedure at the time of these investigations instead of an isolation procedure to determine the presence or absence of tomatine. The assay of extracts of several diseased plants was complicated by the presence of a marked increase in stimulatory substances which may have obscured any slight antifungal activity. Another possibility was that tomatine may have been hydrolyzed by enzymes to its aglycone,

tomatidine, which, although it has as much antifungal activity as tomatine, might not have been extracted from the plant. Recently, we have supplied tomatine in phosphoric acid salt form to plant pathologists for further investigations on plant disease resistance.

Antifungal, Insecticidal, and Growth Stimulating Properties.--The *in vitro* antibiotic activity of crystalline tomatine is given in Table V (9); the activity of tomatidine on an equimolar basis is essentially the same as that found for tomatine. Tomatine has little, if any, antibacterial activity but is reasonably effective against certain of the pathogenic fungi that cause diseases in animals but relatively less effective against fungi pathogenic on plants. Because of the interest in a good antifungal agent for the treatment of systemic fungus infections in man and animals, tomatine and degradation products have been supplied to a number of research institutions for evaluation.

Table V

Comparative Study of the Antibiotic Effect of Tomatine on Microorganisms (9)

Microorganism	Concentration of tomatine, mg./ml. medium [a/]						
	1.0	0.5	0.25	0.1	0.05	0.01	0.0
Achorion gypseum, ATCC 6286	-	-	-	8.0	9.0	14.0	25.5
Achorion schoenleini, ATCC 4822	-	-	-	-	8.0	11.0	15.0
Blastomyces dermatitidis, Duke 1035	-	-	-	-	5.0	11.0	18.0
Epidermophyton floccosum, ATCC 9646	-	-	-	3.0	4.0	7.0	20.0
Microsporum audouini, E 239	-	-	-	-	4.5	9.5	21.0
Trichophyton mentagrophytes, ATCC 9533	-	-	4.5	5.0	6.0	11.0	22.0
Fusarium oxysporum f. *lycopersici*, W R-5-6	14.0	14.5	15.0	15.5	17.0	24.0	32.0
Penicillium notatum, NRRL 124B21	8.0	8.5	9.0	10.0	11.0	13.0	14.0
Candida albicans, Duke 1036	-	-	-	-	+	2+	5+
Candida albicans, E 3147	-	-	-	±	2+	3+	5+
Escherichia coli, NRRL B210	4+	4+	5+	6+	6+	6+	6+
Staphylococcus aureus, NRRL B313	+	2+	3+	3+	3+	3+	3+

a/ The results are expressed as mm. diameter of growth or as plus (+) for growth and minus (-) for no growth.

Drs. Norman F. Conant and Grant Taylor, Duke University School of Medicine, Durham, North Carolina, were the first to test crude tomatine in experimental animals for effectiveness in the treatment of systemic blastomycosis. Crude tomatine proved to be ineffective and quite toxic when given intravenously. Later tests with highly purified tomatine and tomatidine confirmed the earlier observations and, therefore, the toxicity was assigned primarily to the aglycone portion, tomatidine.

Dr. C. W. Emmons, National Institutes of Health, Bethesda, Maryland, has concluded that tomatine is ineffective in the treatment of experimental systemic histoplasmosis. In all, 36 mice were treated. The dose and time interval between doses was varied. The dose varied between 350 and 450 mg. per kilogram and the mice received from 18 to 27 doses. In the case of most of the mice, treatment was given daily. Tomatine was suspended in milk and added to bread and this treated bread was the sole diet of the mice during the treatment period, except in the case of mice which were treated on alternate days or were fed a normal diet on week-ends. The infected mice were not given a lethal infecting dose of Histoplasma, hence no data were obtained on extension of life. The criterion for therapeutic activity was clearance of Histoplasma from organs as determined by culture. Treated animals and controls were killed about one week after treatment was discontinued, and Histoplasma was isolated from all mice.

Dr. John H. Lamb and Gerbert Rebell, Oklahoma Medical Research Institute and Hospital, Oklahoma City, Oklahoma, have tested tomatine and tomatidine against *Blastomyces dermatitidis*. Four strains of *Blastomyces dermatitidis* were tested *in vitro* and both compounds were found to be effective in inhibiting the growth of these organisms at about 0.001% concentration. In view of the *in vitro* effectiveness, they tried to evaluate the *in vivo* effectiveness of tomatine against experimental blastomycosis in mice. Tomatine (5 mg. daily) was administered subcutaneously, in saline suspension, for two weeks to mice inoculated intraperitoneally with *Blastomyces dermatitidis* in gastric mucin. Subcutaneous abscesses were regularly produced at the site of injection of the compound, which appeared not to be absorbed completely from the injection site. There was no evidence of general systemic toxic effect. No effect was observed on the course of the fungus infection. *In vitro* testing of tomatidine by Mr. Rebell against a number of pathogenic organisms gave the following results:

Fungus strain	Test dilutions, % compound				Control dilutions, % acetone		
	0.01	0.001	0.0001	0.00001	4.0	0.4	0
1A3	0	+	+	+++	++++	++++	++++
2A4	+	++	+++	+++	++++	++++	++++
10A1	+	+++	++++	++++	++++	++++	++++
6A7	+	++	++++	++++	++++	++++	++++
7A2	0	0	0	++	++++	++++	++++
9A12	0	+	+	++++	+++	++++	++++
11A2	0	+++	++++	++++	+++	++++	++++
4A4	++++	++++	++++	++++	++++	++++	++++

0 = no growth; + = growth

Key to fungus strains used:

1A3 *Trichophyton mentagrophytes*
2A4 *Microsporum canis*
10A1 *Candida albicans*
6A7 *Cryptococcus neoformans*
7A2 *Blastomyces dermatitidis*
9A12 *Histoplasma capsulatum*
11A2 *Coccidioides immitis*
4A3 *Nocardia asteroides*

Tests conducted by Dr. G. Robert Coatney and associates at the National Institutes of Health, Bethesda, Maryland, have shown tomatine to be ineffective against *Endamoeba histolytica* in guinea pigs when administered orally at dosage level of 50 mg./kg. twice daily for 14 days (6, 31). There was, however, no observed toxicity at this dosage level. Dihydrotomatidine was ineffective against *Plasmodium gallinaceum* in chicks when administered orally at 20 and 100 mg./kg. b.i.d. for four days. It was also ineffective against experimental *E. histolytica* infection in the guinea pig at 40 mg./kg. b.i.d. for five days.

Chick growth experiments were conducted by Dr. H. R. Bird, formerly In Charge, Poultry Investigations, Agricultural Research Center, Beltsville, Maryland. Chickens were fed tomatidine at a level of 3 mg./kg. of diet. It was concluded that tomatidine at this level did not stimulate growth. The level used was approximately three times the level of penicillin and terramycin used in parallel feeding experiments.

Dr. I. E. Wheaton, American Can Company,+ Barrington, Illinois, has concluded that tomatine, tomatidine and dihydrotomatidine do not fall into the classification of successful agents for the control of food spoilage organisms. It is of interest to note, however, that of these three compounds tested, dihydrotomatidine was the most effective as follows:

	Dihydrotomatidine	Tomatidine	Tomatine
Thermophillic flat sour-1518 (tube assay)	50 ppm-negative[a/] 10 ppm-positive[b/]	50 ppm-negative	200 ppm-positive
Putrefactive Anaerobe 3679	100 ppm-negative after 2 months 50 ppm-positive	1, 10 & 100 ppm-positive	75 & 150 ppm-positive
Clostridium botulinum 62A	100 ppm-negative 50 ppm-positive	1, 10 & 100 ppm-positive	75 & 150 ppm-positive

a/ Inhibition of the test organism
b/ No inhibitory activity

A 25 percent dust of tomatine in "Pyrax"+ has been found to be an ineffective insecticide by Dr. R. C. Roark and associates, Agricultural Research Center, Beltsville, Maryland. Insects tested were pea aphid, large milkweed bug, army worm, and two spotted spider mite. A 50 percent dust of tomatine in Kaolin+ at a concentration of 8 pounds to 100 gallons of water, killed 58 percent of European corn borer larvae in 48 hours but at 1 lb. to 100 gallons of water was completely ineffective.

We wish to express our appreciation to the above mentioned investigators for cooperating in this work and for permission to use their data in this report.

Toxicological and Pharmacological Investigations.--Toxicological and pharmacological investigations on tomatine and tomatidine were carried out at the Western Regional Research Laboratory (33). The toxicity studies covered a number of routes of administration. Studies on chronic oral toxicities were made

with rats as the experimental animal, and using both tomatine and its aglycone, tomatidine. Concentrations of these substances, in amounts up to 0.04 percent of the diet, were fed to albino rats for 200 days. This concentration was considered to be well above any amount which might be added to food. Growth rate, health and general appearance of both males and females were normal for a period of 200 days. No abnormalities were seen at autopsy and organ weights were normal. Histological examination of the tissues revealed no abnormalities.

Response to intravenous administration of tomatine was much more dramatic. White mice were used for determining the intravenous LD_{50}. When tomatine was dissolved in propylene glycol, the LD_{50} was found to be between 5 and 10 mg./kg. However, the amount of glycol required to dissolve the tomatine in a volume suitable for injection was very close to an acutely lethal amount of propylene glycol. The tomatine was, therefore, dissolved as the hydrochloride in physiological saline. The pH was less than 3. However, as judged by the control animals, the degree of acidity required for solution of the tomatine was not harmful. The LD_{50} with this preparation was approximately 18 mg. of tomatine/kg. Death, when it occurred, was preceded by a struggle for air and, frequently, a bloody nasal discharge. Death occurred within a few minutes; with the higher doses, death was within seconds. Autopsy revealed severe hemorrhage in the lung tissue. Except for an early, slight depression, survivors showed no symptoms during the several days of observation.

The symptoms just described were explained by the following experiments: White rats under sodium pentobarbital anesthesia were used for kymographic recordings of carotid blood pressure. Injections, except for the initial intraperitoneal dose of anesthetic, were by cannula into the external jugular vein . Doses of 0.5 to 2 mg. of tomatine/kg. caused an immediate, severe drop in blood pressure. In some animals, recovery to normal pressure was equally rapid, and repeated injections over a period of 3 or 4 hours, up to the death of the animal, produced similar reactions. In other animals, recovery was slow and the pressure sometimes never regained its original level. Preliminary results indicated that the effect was mediated through the vagi, since ligation of these nerves or, some times, atropinization, prevented the drop and occasionally substituted a slight rise in blood pressure. The sudden death of the mice could be explained by the precipitous drop in blood pressure.

After a series of injections, hemoglobinuria was sometimes observed. *In vitro* studies with rat blood, showed that some hemolysis could be observed with 0.4 mg. of tomatine/100 cc. of blood. The hemorrhagic lungs and the bloody nasal discharge probably can be correlated with this hemolytic action.

An incidental reaction was observed and is recorded without further study. A clot formed when tomatine was added to citrated blood, the amount of clot increasing with an increase in the concentration of tomatine. When sodium fluoride was the anticoagulant, clotting did not occur. This might suggest that tomatine combined with the citrate, thus freeing calcium.

Subcutaneous injections of tomatine as the hydrochloride or in propylene glycol produced an undesirable local reaction without observable systemic effects. Ten to 40 mg. of tomatine/kg. in rats produced subcutaneous abscesses, the larger abscesses tending to ulcerate.

Topical application of tomatine produces a minimum of reactions. A five percent ointment of tomatine in Carbowax+ was used in these studies. For studying the effects on highly sensitive tissue, the rabbit's eye was utilized. Approximately 300 mg. of the ointment was placed in one eye of each animal on 9 of 11 days. The ointment base was put in the opposite eye. Tomatine proved to be irritating, causing conjunctivitis within 24 hours. As the treatment progressed, the inflammation became progressively worse. The palpebral conjunctive seemed to be the more severely affected and the lid became edematous. Four to five days after discontinuing treatment the eyes appeared normal. The control eyes were normal at all times.

Action on the skin was studied in rats, rabbits and guinea pigs. The ointment was rubbed onto the skin of rabbits, near the shoulder, at the same time as applications were made to the eyes. Little or no erythema was noticeable during the 11 days of the treatment period. To test this further, and to observe possible systemic reactions, rats were shaved from neck to hips and the ointment was applied liberally to this area once a day for 10 of 12 days. The skin appeared normal at all times. Gross observation revealed no abnormal reactions in these rats. In an attempt to see if any of the tomatine was absorbed, periodic erythrocyte counts and hemoglobin determinations were made. This was done because of the previously discussed hemolytic action of tomatine. No consistent trends were noted. Repeated intradermal injections of tomatine in rabbits and guinea pigs did not produce a skin sensitization.

Gastric administration to rats of 900-1000 mg. of tomatine/kg. led to depression terminated by death after some 24 hours. The stomach was distended with twice as much fluid as had been given by stomach tube. Presumably there was spasm of the pylorus, probably due to the irritant action of tomatine on the mucosa. That some of the tomatine had been absorbed is suggested by the peculiarly hemorrhagic appearance of certain tissues (pancreas, adrenal, spleen), probably due to the hemolyzed blood.

This acute dosage, 1 gram/kg. is definitely excessive. A quarter of this, 250 mg./kg., was given to a series of rats daily for five days with no notable adverse reactions. And, as noted earlier, diets containing 0.04 percent tomatine have not been harmful when eaten for 200 days. This would be a daily intake of 15-20 mg. of tomatine/kg.

This work suggests that the oral administration of tomatine is safe for all reasonable dosage levels. Application in ointment to skin appears acceptable. The irritation to more sensitive tissues is not permanent and perhaps could be eliminated by suitable dilution. Parenteral administration is inadvisable. Details of these investigations will be published elsewhere.

FUTURE POSSIBILITIES FOR TOMATINE AND RELATED STEROIDAL ALKALOIDS

The results of experiments given in the preceding sections suggest possible commercial outlets for tomatine and derived products as (a) antifungal agents in the treatment of mycotic skin diseases (b) precursors for sex and other hormones and (c) precursors for other physiologically active compounds wherein the nitrogen containing portion of the molecule is not completely removed.

The possible use of tomatine, tomatidine and derived products as antifungal agents is receiving some attention. One of the problems has been the commercial availability of tomatine. There is as yet no known commercial supply produced in the United States, however, Drógaco Industria Quimica S.A.,† Buenos Aires, Argentina, offered tomatine for sale (3) in January 1955. In a four page pamphlet entitled "Tomatina" (3) it was reported that clinical experience in Argentina with a hydrophil ointment of 0.5 percent tomatine proved its usefulness in mycotic dermatosis of any form. Tomatine was especially recommended for diseases, such as Eczema, Tinea trichophytina, Tinea circinata, Tinea favosa, Favus of body and nails, Tinea microscopic, Intertrigo and onyx blastomycetes, Pityriasis versicolor and Erythrasma. The antimycotic preparations of tomatine were reported to be odorless, did not cause local irritations and gave no symptoms of secondary toxicity or cutaneous sensitivity. Tomatine was recommended for use in the form of an aqueous ointment, powder or lotion at 0.5 percent concentration. In all liquid preparations the use of a buffer of pH 4.5 to 5.0 was necessary to solubilize the drug. The consistency of the ointment was adjusted by using synthetic gums (methyl or carboxymethyl cellulose), which do not precipitate the glycosidal alkaloid.

It is stressed here that as yet no clinical data have been published in the United States to substantiate the above report (3). Whether or not tomatine can be produced economically in the United States remains to be determined.

As a precursor of sterols, tomatine is easily degraded to tomatidine and then to a pregnene derivative (9, 28). Tomatidine is not at present a "chemical of choice" in the conversion to cortisone and other hormones because of one structural limitation. Our work has pointed up, however, the great potential reservoir of new useful alkaloids in solanaceous plants. Prior to our work, it was stated that tomato plants contained solanine, an alkaloid found in the Irish potato plant (*Solanum tuberosum* L.) We have not found solanine in tomato plants. Work on other solanaceous plants in New Zealand (4), paralleling ours, has shown that there is present in certain *Solanum* species an alkaloid, *solasodine*, closely related to tomatidine which has all the structural requirements necessary to make conversion to cortisone and other hormones as easy as from diosgenin. The key structural requirement in solasodine and diosgenin that is missing in tomatidine is a Δ^5-double bond. Solasodine is known to occur in glycosidic form in *Solanum sodomeun* L., *S. auriculatum* Ait., *S. marginatum* L.f. and *S. aviculare* Forst. f. (24, 26, 30) and has been degraded to 3 (β)-acetoxy-$\Delta^{5,16}$-pregnadiene-20-one (29). Solasodine is obtained from leaves and fruit of these plants, in contrast to a root crop for the sapogenin, diosgenin. It would appear that the growth requirements of these *Solanum* species might be investigated to advantage in the United States from the standpoint of developing a domestic precursor source for cortisone and other hormones and a new crop for farmers.

Chemical modifications of tomatidine, in which the nitrogen portion of the molecule is not completely removed, have been achieved. A number of new compounds that will warrant pharmacological investigation have been prepared.

Tomatine and tomatidine have not been eliminated as possible contributing factors in determining the degree of disease resistance of tomato species to fusarium wilt. Additional work on this important problem needs to be done by a team of chemists and plant physiologists and pathologists.

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